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Seasonal dynamic and in situ division rates of the dominant *Dinophysis* species in Punic harbors of Carthage (Gulf of Tunis, South Mediterranean)

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Abstract This study investigates spatio-temporal variations of in situ division rate (μ) of two species of the genus *Dinophysis*. Based on a postmitotic index approach, estimates of μ from net-haul samples were compared during five daily cell cycles studies in three stations of the Punic harbors of Carthage (Gulf of Tunis). To study the annual cycle of *Dinophysis* spp., weekly sampling were carried out from March 2008 to June 2010. In addition, sampling over 24 h was conducted on three-cycle studies in late spring and two-cycle studies in autumn, seasons characterized by *Dinophysis* proliferations. We recorded important abundances of *Dinophysis sacculus* stein (2.25×10^4 cells/l, June 2008) and *Dinophysis* cf. *acuminata* (3×10^4 cells/l, June 2009). Cellular division was phased in *D. sacculus* stein and *D.* cf. *acuminata*, but the division time, required for the population cohorts to pass from one phase to another, and values of estimated division rates varied between seasons and stations for the same species. *D.* cf. *acuminata* exhibit moderate (0.22 day^{-1}) to high (0.68 day^{-1}) value of μ. In contrast, *D. sacculus* shows very low μ values (0.02–0.17 day^{-1}) in autumn season to moderate values (0.21–0.35 day^{-1}) in late spring. The results presented here confirm that the postmitotic index approach allowed estimated of μ at low field depths and concentrations (10^2–10^3 cells/l).

Keywords *Dinophysis* spp. · *In situ* division rate · Mitotic index approach · Low field depths

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Introduction

Dinophysis species have been well documented in the western and eastern Mediterranean Sea by many works describing their morphology, biology, and ecology (Nikolaidis and Moustaka-Gouni 1990; Cabrini et al. 1993; Delgado et al. 1996; Zingone et al. 1998; Aubry et al. 2000; Koukaras and Nikolaidis. 2004; Batifoulier et al. 2012) and discussing about their toxicity (Lassus et al. 1985; Bravo et al. 1995; Marasović et al. 1998) and the phylogeny of several species of this genus (Papaefthimiou et al. 2010). In contrast, to the south Mediterranean Sea (Northern African coasts), documentation about *Dinophysis* spp. are few; indeed, monitoring surveys were conducted on toxic marine phytoplankton, especially in Morocco (Bennouna et al. 2000; El Madani et al. 2011), in Algeria (Frehi et al. 2007), and in Tunisia (Turki 2004; Armi et al. 2008) showing the presence and proliferations of *Dinophysis* species. Kacem et al. (2009), and Armi et al. (2011) recorded *Dinophysis sacculus* blooms associated with

diarrheic shellfish intoxication in Bizerte and Tunis north lagoons in the north of Tunisia. Several species of *Dinophysis* are regular members of phytoplankton assemblages in the coastal waters of the Gulf of Tunis. *Dinophysis* cf. *acuminata* and *D. sacculus* are the most abundant species with maximum annual concentrations ranging from 5.2×10^3 cells/l (Turki 2004) to 4.6×10^4 cells/l (Armi et al. 2011) in Tunis north lagoon. According to Turki et al. (2006), these species are very persistent and represented 41.7 % of Bizerte lagoon dinoflagellates. Considering the socioeconomic impact of frequent proliferations of *Dinophysis* spp. causing shellfish harvesting closures in the north Tunisian coastal waters, such as in Tunis north lagoon in January and February 1997 and also in February, April, and December 1999 (Turki 2004).

Frequent proliferations of *D. sacculus* are associated with diarrheic toxins detected in clams and mussels in the north Tunisian coastal waters, such as in Tunis north lagoon in January 2007 (Armi et al. 2011) and in Bizerte lagoon during 2006 (Kacem et al. 2009).

The estimate of in situ division rate of these species is essential to analyze the initiation of *Dinophysis* population and the hydrographic conditions that advance either potent in situ division or accretion resulting from physical/biological interactions.

In the growth equation, the specific division rate, μ, represents the most essential intrinsic parameter that needs to be determined. This parameter estimates the potential for intrinsic division without the interference of losses attributable to grazing, mortality, and physical dispersion (Carpenter and Chang 1988). In situ specific growth rate measurements in natural phytoplankton populations are sparse (Stolte and Garcés 2006), particularly in the case of *Dinophysis* spp., which often occur in low densities and entrenched in phytoplankton assemblages dominated by other species. Estimates of μ in field populations of *Dinophysis* spp. have been obtained either from incubations, on deck, or in diffusion chambers (Garcés et al. 1997), or from direct in situ high frequency sampling with a mitotic index approach (Reguera et al. 2003; Velo-Suárez et al. 2009). Among these species, *D.* cf. *acuminata* was the first successfully cultured species of the genus (Park et al. 2006), but trials to grow *D. sacculus* are still ongoing. However, its growth rate in natural population was studied by Garcés et al. (1997).

Two previous studies were based on postmitotic index approach, the first estimated in situ division rates of *Dinophysis* spp. at different depths on different seasons (Reguera et al. 2003), but the second compared values of μ estimated from integrated water-column samples with values from samples collected at the depth where the maximum concentration of *Dinophysis* spp. was detected (Velo-Suárez et al. 2009). In the same framework, the present paper presents for the first time the annual cycle of *Dinophysis* species in the Punic harbors of Carthage (North African Coasts of Mediterranean Sea) and compares estimated values of in situ division rates (μ) of *D. sacculus* and *D.* cf. *auminata* in three different stations at low depths in different seasons.

Material and methods

Study area

The Punic harbors of Carthage (36°50′ N 10°19′ E) are located in the southern area of the Gulf of Tunis at the proximity of Tunis north lagoon and Rades harbor. They are composed of two basins that cover an area of about 8 ha, communicating with each other and with the Bay of Tunis by channels (Fig. 1). These coastal basins represent a shallow water ecosystem with an average depth of 3.20 m. They are very enclosed with small exchanges with the bay of Tunis and are considered to be eutrophic due to increased nutrient loading caused by a rapidly expanding human population in this region since 1990s. According to Souissi et al. (2000), the west coast of the Gulf of Tunis is an eutrophic area. They noted a relative nutrient enrichment in this zone due to the urban and industrial discharges through Rades channel, besides to the harbors (Rades and la Goulette) and terrestrial inputs influence.

Field sampling

The present study is based on samples from 5 diel cycles studies carried out between 2008 and 2010 in three stations of the Punic harbors of Carthage, essentially in early summer (3 and 4 June 2008; 15 and 16 June 2009; and 26 and 27 June 2010), and mid-autumn (2 and 3 November 2008; 30 and 31 October 2009) matching with numerical increase of *Dinophysis* spp. (Aissaoui et al. 2007).

Because of the shallow depths in the Punic harbors of Carthage, samples were collected by vertical net

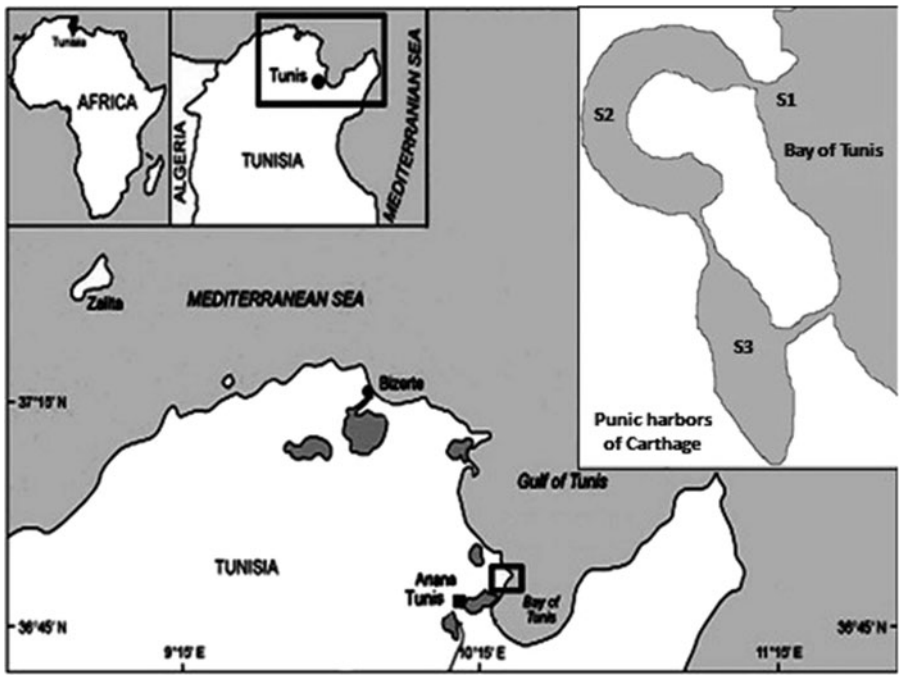

Fig. 1 Site of sampling

hauls with a 20-μm mesh from the whole water column in three stations: station S1 (mean depth, 5.5 m) is the point of water exchange between the Gulf of Tunis and the north basin (larger than the southern point of communication), station S2 (3 m) corresponds to the north basin, and station S3 (2.3 m) represents the south basin. Simultaneously, temperature and salinity were measured at each station. The Chl-*a* was extracted in 10 ml of 90 % acetone for 24 h, in the dark at −4°C, and the extract concentration was analyzed spectrophotometrically (UV-visible spectrophotometer PU-8800).

Vertical haul samples were further passed through a 150-μm mesh to eliminate debris and large zooplankton organisms and immediately fixed with Neutral Lugol's iodine solution (Andersen and Throndsen 2004).

Spring and autumn cycles were carried during 22 and 26 h. Vertical net-haul samples were collected hourly from 1100 to 0500 hours and every 30 min from 0500 to 1100 hours [periods when cytokinesis and sulcal list regeneration can take place rapidly (Reguera et al. 2003)].

For the annual cycle study of *Dinophysis* spp., weekly samples were collected by vertical 20-μm mesh net hauls in each station. From all Lugol's fixed samples cells, *Dinophysis* spp. were counted by the Utermöhl (1931) method using 25 sedimentation chambers and IMT2 inverted Olympus microscope (the total bottom of the sedimentation chamber is scanned at ×200–×400 magnification). Some specimens were observed under epifluorescence microscopy (Porter and Feig 1980) using DAPI (Sigma) stain (filtration of 100 ml of sampling water through 0.22-μm pore size filter and addition of about 200 μl of DAPI solution).

Estimates of division rates (μ)

According to Reguera et al. (2012), the potential growth rate (sensu Carpenter and Chang 1988), estimated by the mitotic index approach or the specific growth rate of most authors, is μ_{gross} and results from cell division only and will be maximal under most favorable physical conditions and availability and accessibility of resources. In contrast, the net growth rate (μ_{net}) depends on cell divisions, mortality due to grazing, parasitism and lyses, immigration through physical accumulation, and aggregation and emigration from physical dispersion or sinking. This mitotic index approach of estimating growth rate has been the most widely used method to estimate μ_{gross} of *Dinophysis* spp. in natural population through high frequency

sampling of the cell cycle. This method is based on the morphological recognition and quantification of terminal events in cells undergoing mitosis.

Estimates of in situ division rates were based on the mitotic index approach, calculated following the model of Carpenter and Chang (1988), using the frequency of dividing (paired) and recently divided (incomplete development of the left sulcal list) cells, which were recognized by their distinct morphology as described in Reguera et al. (2003):

$$\mu = \frac{1}{n(T_c + T_r)} \sum_{i=1}^{n} (t_s) i \ln[1 + f_c(t_i) + f_r(t_i)] \quad (1)$$

where μ is the daily mean specific division rate, $f_c(t_i)$ is the frequency of cells in the cytokinetic (or paired cells with incomplete development of left sulcal list) phase (c), and $f_r(t_i)$ is the half frequency of cells in the recently divided (missing the lower part of the left sulcal list) (r) phase in the ith sample. T_c and T_r are the duration of the c and r phases, considered as terminal events (sensu Carpenter and Chang 1988) in the present study; n is the number of samples taken in a 24-h cycle; and t_s is the sampling interval in hours. The duration of the selected terminal events, T_c+T_r, recently defined as division time (T_D) (Reguera et al. 2012), time required for the cohort of cells to pass from one phase to the next. In this case, the time interval between time t_0, when the frequency of cells undergoing cytokinesis (f_c) is maximum and time t_1, when the fraction of recently divided cells f_r is maximum:

$$\frac{1}{2}(T_c + T_r) = (t_0 - t_1) \quad (2)$$

where T_c, T_r, t_1, and t_0 are calculated after fitting a fifth degree Gaussian function to the frequency data.

For the estimation of a minimum division rate during the 24-h cycle, we used the maximum frequency approach (McDuff and Chisholm 1982). This approach supposes the possibility of recognizing all the dividing and recently divided cell in a given day in one sample.

$$\mu_{min} = \ln(1 + f_{max}) \quad (3)$$

where f_{max} is the maximum frequency (f) of dividing cells (paired cells plus recently divided cells) was estimated using the equation:

$$f_{max} = \frac{p + \frac{I_r}{2}}{I_c + p + \frac{I_r}{2}} \quad (4)$$

where p=paired cells, I_c=fully developed individuals, and $I_r/2$=50 % of recently divided cells (I_r).

Data were log transformed, and a Pearson correlation was applied in order to estimate regular relations between *D. sacculus* and *D. acuminata* abundances and transitions of the two abiotic factors temperature and salinity. This study is supported using conventional methods on the basis of Excel programs and STATISTICA software, version 6.0 (Stat Soft, Inc.).

Results

Hydrographic parameters

During our survey in Punic harbors of Carthage, temperature ranged between 11.6 °C in February 2008 and 30 °C in August and September 2009. There was no high variation in temperature between the three stations; however, the highest temperature value (30 °C) is usually recorded in the station S3. We recorded an increase of water temperature of 5 °C in spring and early summer and a fall in temperature of 10 °C in mid-autumn (Fig. 2). During these periods, we observed *Dinophysis* proliferations. High variations in salinity [40.30 (August 2008)–32 (December 2009)] were registered in Punic harbors of Carthage in the station S2, which is situated in north basin which has an arc shape. In the stations S1 and S3, the lowest salinity values were recorded on May (33.50), and the highest values were observed on September 2009 (39) (Fig. 3).

We noted that heterogeneous profiles of temperature and salinity were observed during different intensive sampling diel cycles:

Diel cycle 1 (3 and 4 June 2008) This period was characterized by an increase in water temperature and salinity (Fig. 2). During sampling, mean hourly temperature in the Punic harbors of Carthage ranged from 15.13 °C at 0400 hours to 27.20 °C at 1300 hours (Fig. 2). These values show a difference of 12 °C noted especially in the station S2.

The highest salinity (36.70) was detected at 0500 hours, and the lowest one (35.40) was recorded at

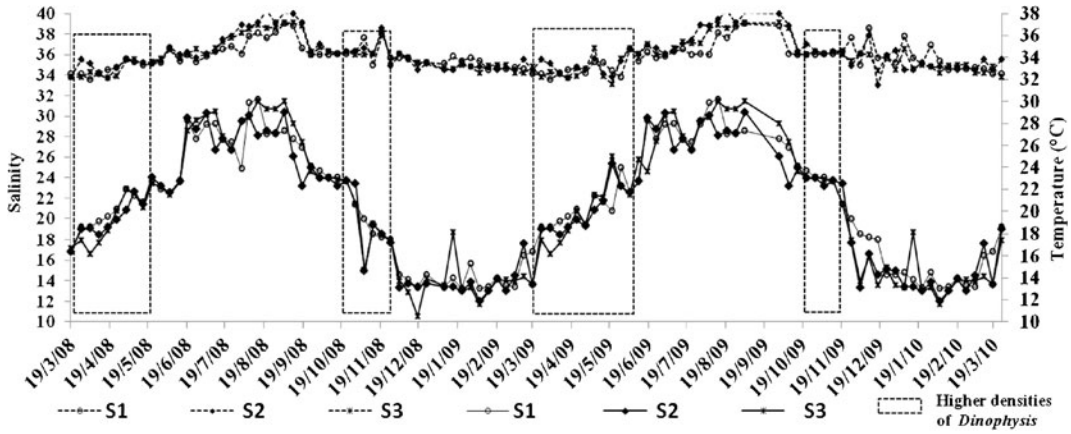

Fig. 2 Spatiotemporal variation of water temperature and salinity in Punic harbors of Carthage from March 2008 to March 2010

1600 hours in the same station S3. Figure 3 shows that salinity values were maximal during the hours just before sunrise and decline after. The phytoplankton was mainly formed by armored dinoflagellates (*Coolia monotis*, *Alexandrium catenella*, and *Scrippsiella* sp.) and centric diatoms (*Chaetoceros* sp., *Pleurosigma obscurum*, and *Thalassiosira rotula*), and there was a dominance of *D.* cf. *acuminata* and *D. sacculus* at the dark period of the diel cycle in the three stations. *Dinophysis rotundata* and *Dinophysis caudata* were observed rarely. *D.* cf. *acuminata* was the most abundant dinoflagellate. Maximum concentrations of this species were found from 0300 to 1100 hours (Fig. 3).

Diel cycle 2 (2 and 3 November 2008) One week before this cycle sampling, a decrease in water temperature and salinity was registered following a heavy rain. Hourly surface water temperature varied slightly from 18.1 °C at 1800 hours to 20.50 °C at 0800 hours and rising steadily to about 26.77 °C at 1300 hours (Fig. 4). High salinity values were recorded during early morning before sunrise reaching 36.40 at 0600 hours. Lower water salinities (34.70–35.80) were registered the following day in the station S1. Phytoplankton samples were composed of filamentous cyanobacterial species (*Oscillatoria* sp), diatoms (*Pleurosigma directum* and *Navicula* sp.), and dinoflagellates species (*Alexandrium catenella* and *Prorocentrum triestinum*). *D.* cf. *acuminata* and *D. sacculus* were the most frequently species. *D.* cf. *acuminata* was the dominant dinoflagellate especially at night samples (Fig. 4). However, densities of *D. sacculus* were very low ($1–7\times10^2$ cells/l) at night and increased sharply after sunrise reaching 4.20×10^3 cells/l at 1030 hours (Fig. 4).

Diel cycle 3 (15 and 16 June 2009) From 1900 to 0900 hours, surface water temperature varied slightly. A distinctive increase in temperature values was detected during following hours reaching 29 °C at 1200 hours (Fig. 5). High salinities were recorded during the last hours of the night up to 37.4. Through the rest of the day, slightly variations of water salinity were observed. Phytoplankton was dominated by high concentrations of *D.* cf. *acuminata* and *D. sacculus* accompanied by important proliferation of *C. monotis* and low densities of *D. rotundata* and *D. caudata.*

Diel cycle 4 (30 and 31 October 2009) Few days before this date, we noted a decline of temperature and salinity values (Fig. 2). Temperature varied between 18.30 and 20 °C during dark period with a smooth increase during light period (Fig. 6). Water salinity shows low values at early night; nevertheless, maximum values were recorded at hours just prior to sunrise (0642 hours; Fig. 6). Many phytoplankton species were observed, among which *Oscillatoria* sp, *Alexandrium catenella*, *Akashiwo sanguinea*, *Chaetoceros* sp., *Lingulodinium polyedrum*, and *Prorocentrum lima*, were the most frequent in all water samples. *D. sacculus* and *D.* cf. *acuminata* were also present with important concentrations. *D.* cf. *acuminata* was the most abundant during dark period of the diel cycle, while, *D. sacculus* proliferated more during light period. In contrast, *D. caudata* was generally rare (30–300 cells/l).

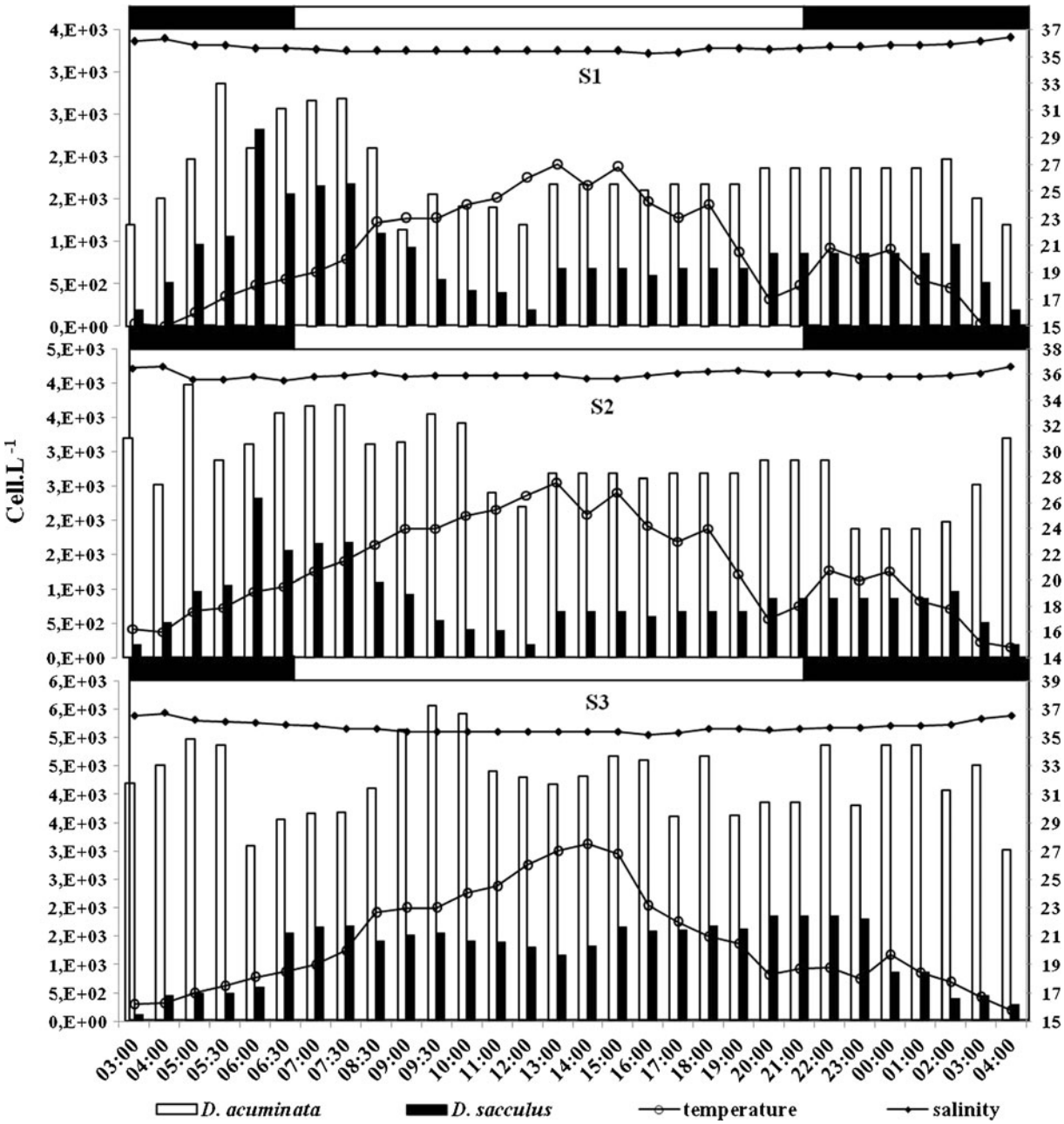

Fig. 3 Variation of temperature, salinity and abundances of *D.* cf. *acuminata* and *D. sacculus* in Punic harbors of Carthage during sampling cycle of 3 and 4 June 2008

Diel cycle 5 (26 and 27 June 2010). High surface water temperature and salinity were recorded in this period every year of the survey (Fig. 2). Temperature water ranging between 24.70 and 25.30 °C from 2000 to 0900 hours and values increased gradually reaching 29.70 °C at 1330 hours (Fig. 7). At the beginning of a night of full moon, salinity varied slightly and increased sharply in the end of dark period, reaching 38 psu at 0300 hours. Only few dinoflagellate species were present in phytoplankton samples such as *C. monotis*, *L. polyedrum* and *A. sanguinea*. *Dinophysis* species (*D.* cf. *acuminata* and *D. sacculus*) were the dominant. Low densities of *D. sacculus* (2×10^2 to 4×10^2 cells/l) were recorded during night and moderated to important

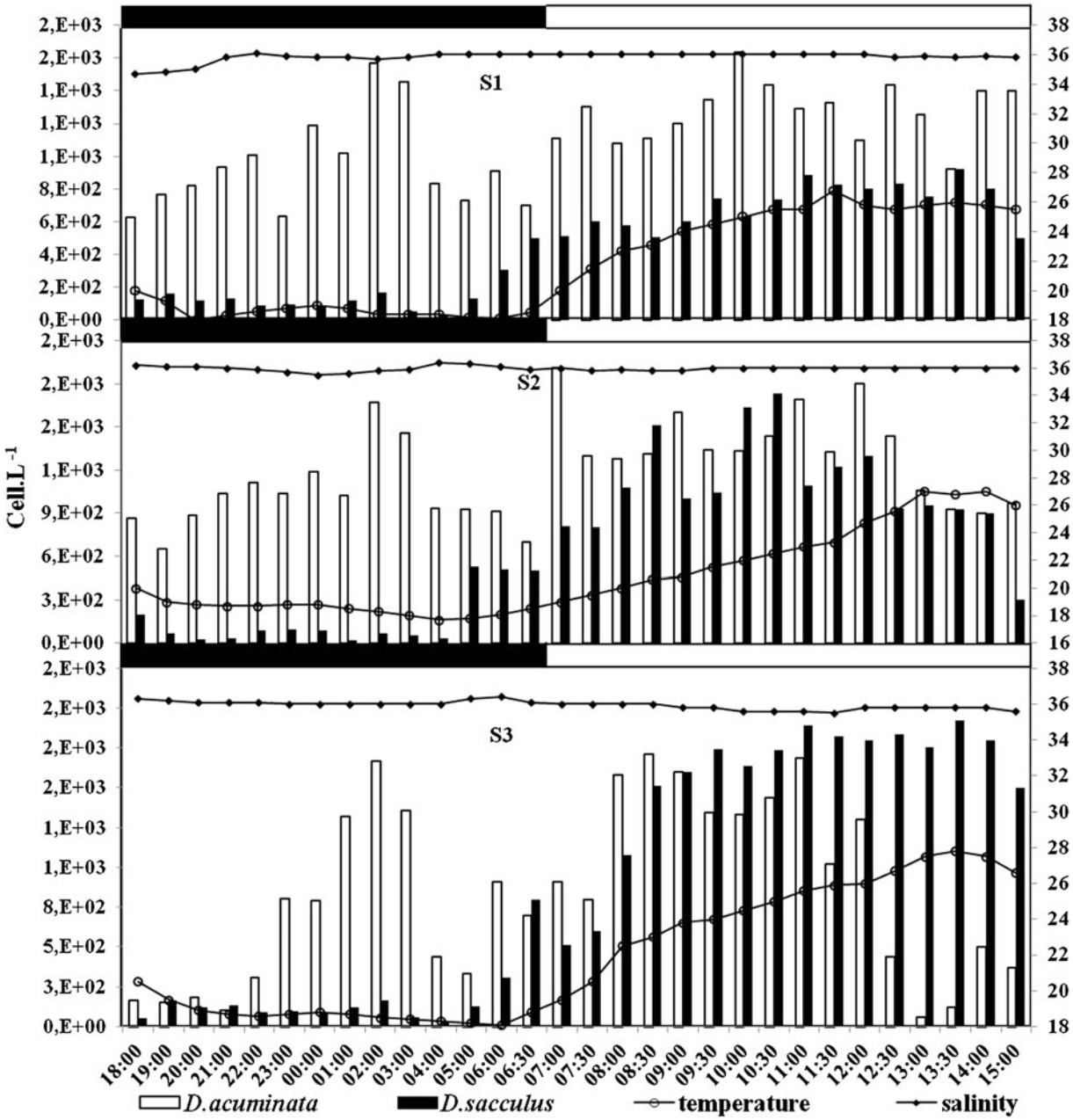

Fig. 4 Variation of temperature, salinity and abundances of *D.* cf. *acuminata* and *D. sacculus* in Punic harbors of Carthage during sampling cycle of 2 and 3 November 2008

concentrations (1.10×10^3 to 7.40×10^3 cell/l) in samples collected following sunrise (Fig. 7). Important proliferations of *D.* cf. *acuminata* were observed in dark period (3.80×10^3 to 7.60×10^3 cells/l) and one peak (11.30×10^3 cells/l) was recorded at 1330 hours. *D. rotundata* was uncommon and present only in some samples taken from the stations S1 and S2.

Dinophysis species in Punic harbors of Carthage In Punic harbors of Carthage, high concentrations of Chl-*a* were registered during spring and summer reaching 6.67 μg l^{-1} on June 2009 (Fig. 8). Generally, low values were observed in October–December during the decline of total phytoplankton cell number. The phytoplankton community was mainly represented by

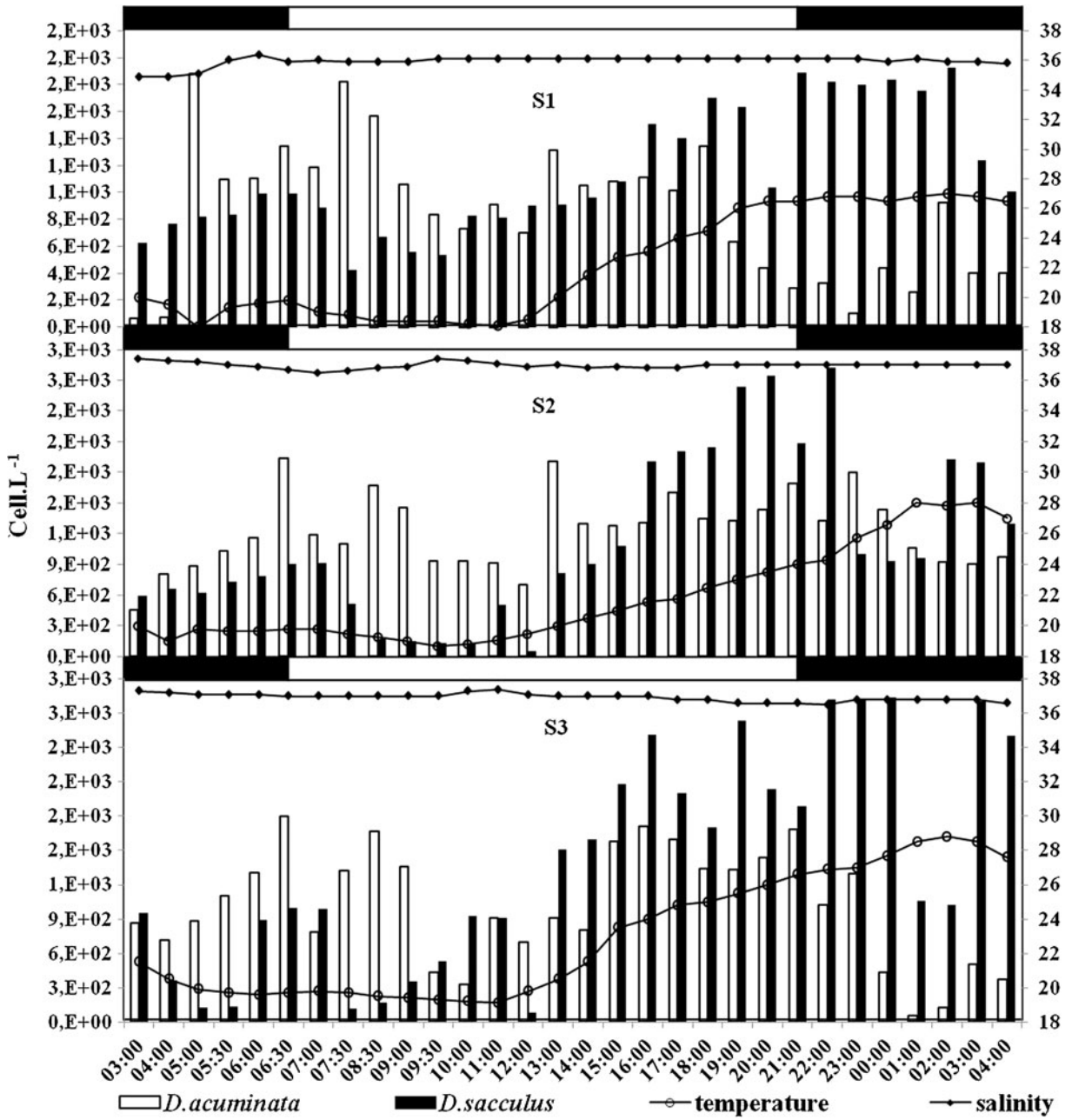

Fig. 5 Variation of temperature, salinity and abundances of *D*. cf. *acuminata* and *D. sacculus* in Punic harbors of Carthage during sampling cycle of 15 and 16 June 2009

dinoflagellates during late spring, summer, and early autumn. Dinophysiales were observed throughout the year with others phytoplanktonic species, but in March and April 2009, we recorded only monospecific proliferations of *Dinophysis* spp. (Fig. 9).

Four species of the genus *Dinophysis* were identified during this study: *D*. cf. *acuminata*, *D. sacculus* Stein, *D. rotundata* Claparède and Lachmann, and *D. caudata* Saville-Kent. The two species, i.e., *D*. cf. *acuminata* and *D. sacculus*, showed a wide temporal distribution throughout the sampling period (Fig. 10). *D*. cf. *acuminata* occurred in water samples from January to the beginning of July and from September to December 2009. High concentrations (1.90×10^4 to

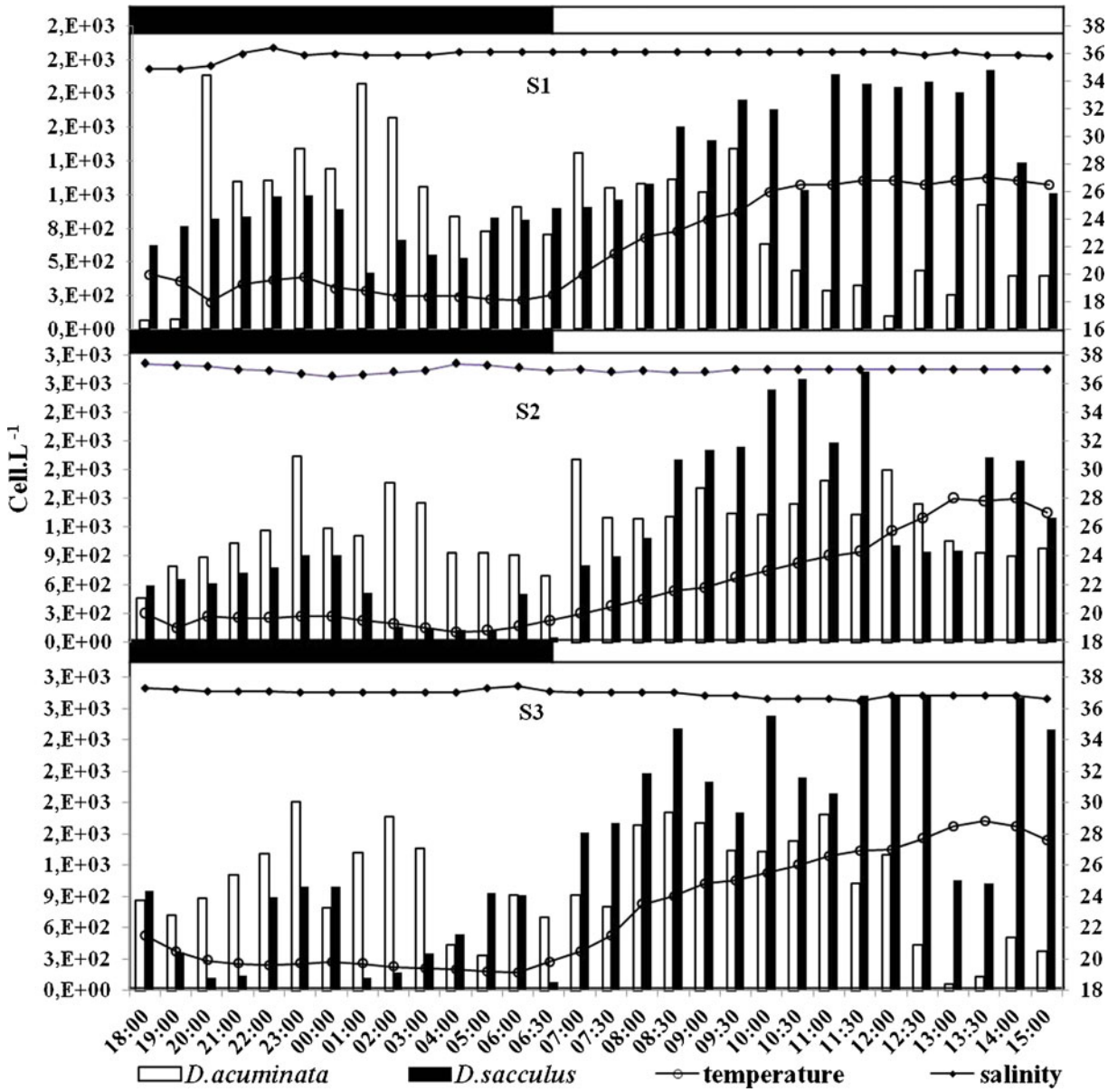

Fig. 6 Variation of temperature, salinity, and abundances of *D.* cf. *acuminata* and *D. sacculus* in Punic harbors of Carthage during sampling cycle of 30 and 31 June 2009

6.30×10^4 cells/l) were recorded from March to June 2008–2009. Other important proliferations were observed from October to November (from 10^3 to 6×10^3 cells/l^{-1}, 2009). This species showed two different morphotypes. The first represented 97 % of spring and winter populations; cells have an ellipsoid form, broadest near the middle part of the cell with a more or less small cingular list (Fig. 11). Cell size varies between 42 and 48 μm in length (44.52±2.52, n=100) and between 27 and 37 μm in width (31.62±2.29, n=100) with a length/width ratio (L/W) ranging from 1.20 to 1.50 (1.41±0.17, n=100). The second morphotype represented over 80 % of early summer and autumn populations. This morphotype presented cells with characteristics fairly close and similar to the original description of *D. acuminata* (Fig. 11).

D. sacculus Stein occurred in the Punic harbors with significant concentrations (3×10^3 to $2.25\times$

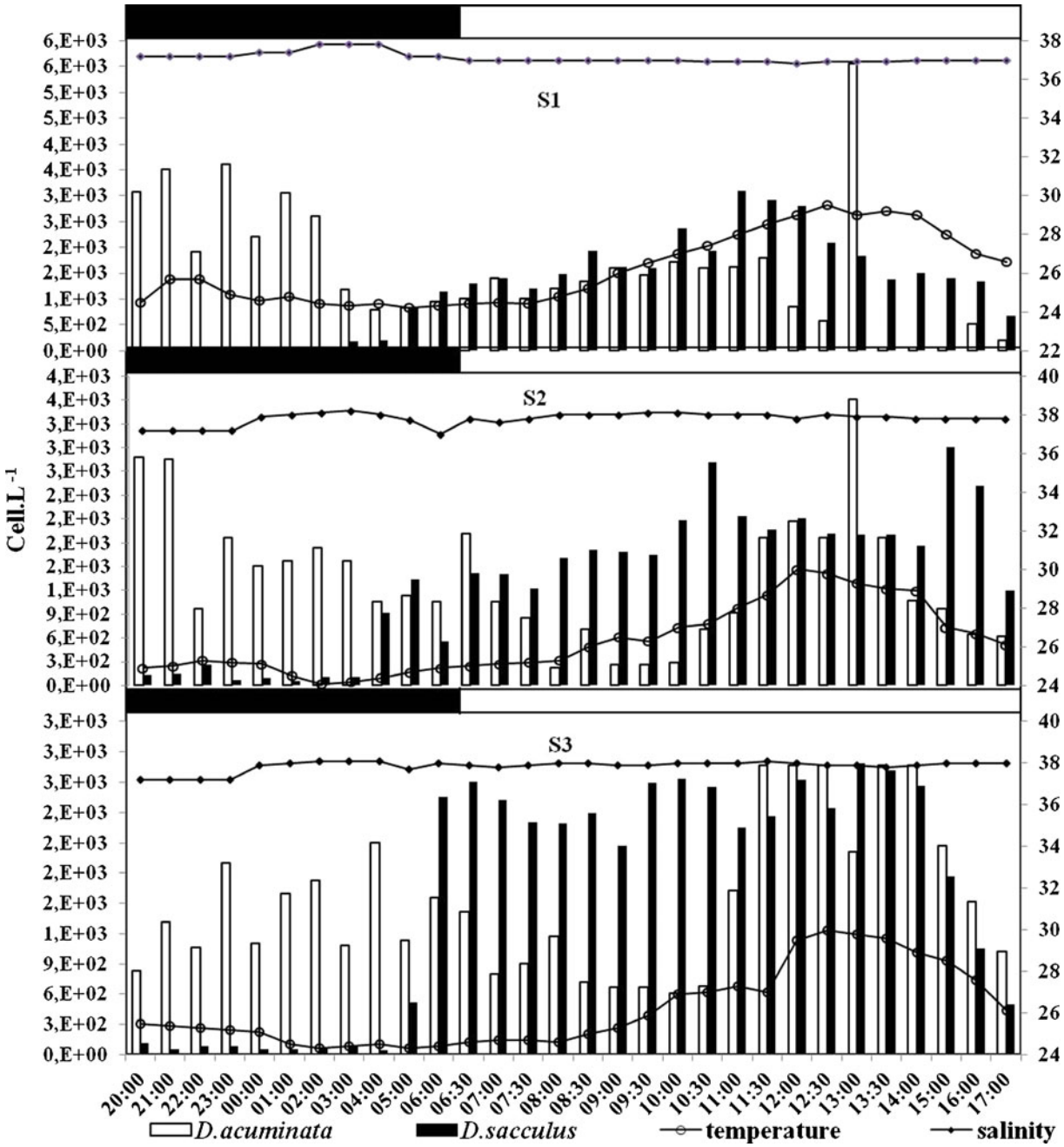

Fig. 7 Variation of temperature, salinity, and abundances of *D*. cf. *acuminata* and *D. sacculus* in Punic harbors of Carthage during sampling cycle of 26 and 27 June 2010

10^4 cells/l) from May to July (2008–2009) low to moderate densities from September to November (2008–2009; 70 cells/l^{-1} to 2.10×10^3 cells/l) and appeared sporadically during the rest of the year (Fig. 10). *D. rotundata* was relatively uncommon (not exceeding 345 cells/l) in stations S1 and S2 on August and September (2008). In the three stations, *D. caudata* was also observed in low densities (≤300 cells/l) during late summer and early autumn (Fig. 10).

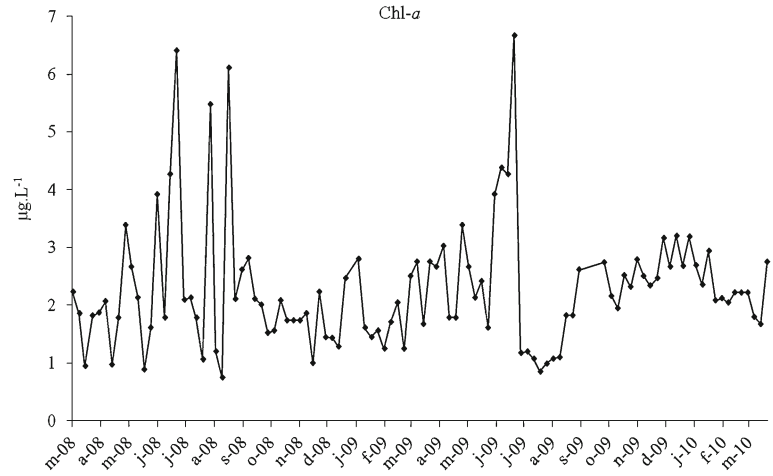

Fig. 8 Chlorophyll-*a* temporal variation in the Punic harbors of Carthage

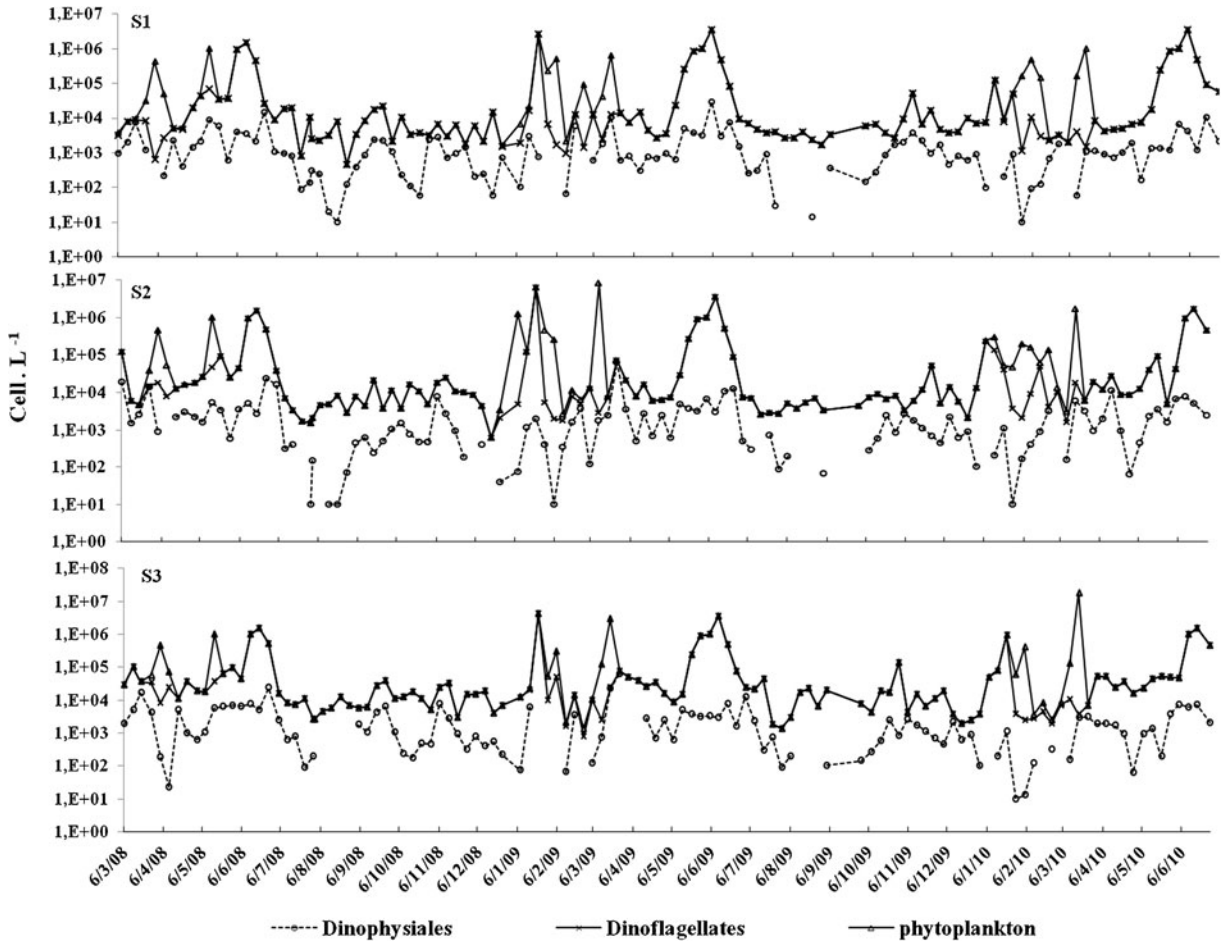

Fig. 9 Spatiotemporal variation of dinophysiales, dinoflagellates, and total phytoplankton from March 2008 to June 2010 in Punic harbors of Carthage

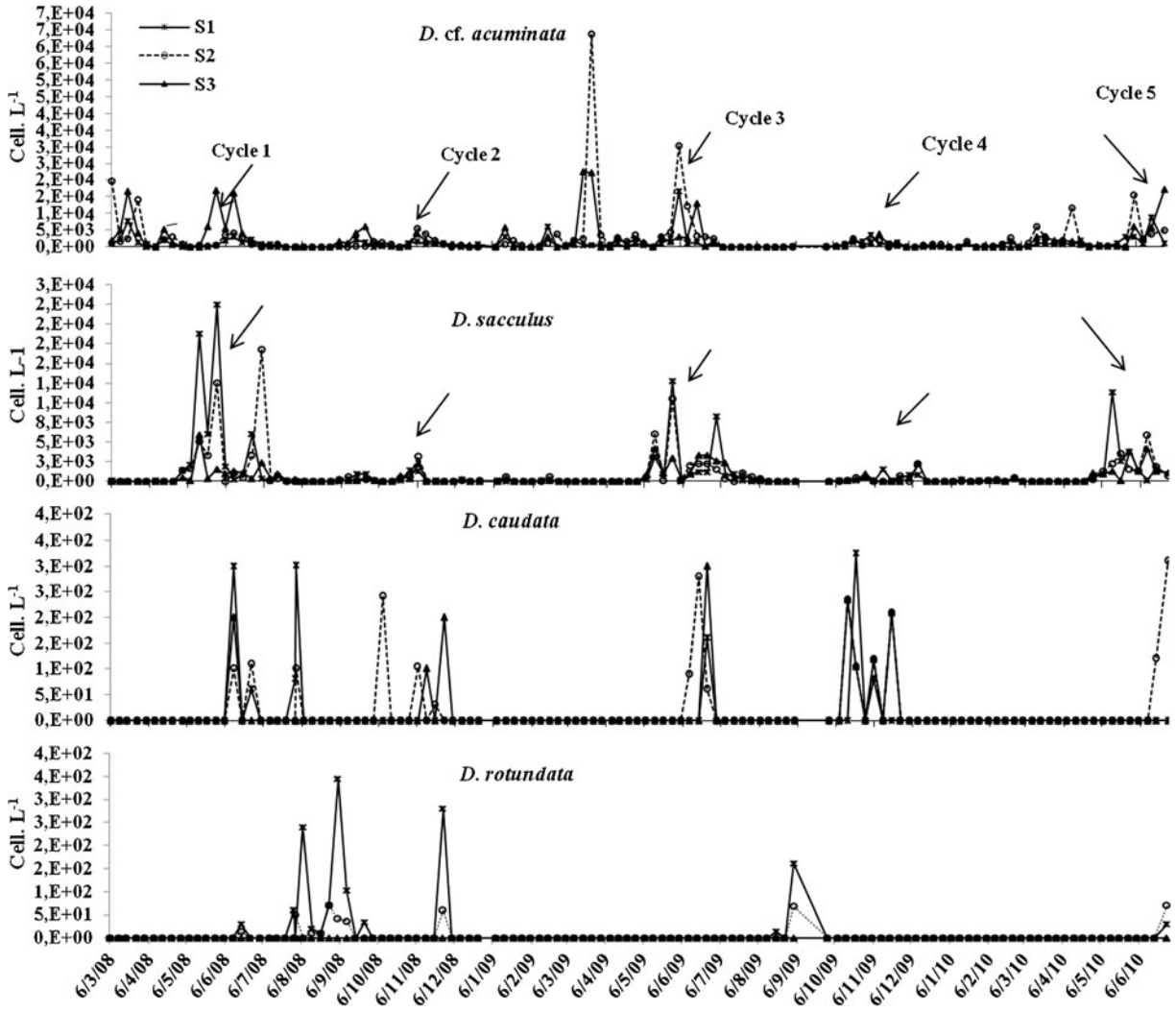

Fig. 10 Yearly distributions of cell concentrations of *D.* cf. *acuminata* and *D. sacculus*. *Arrows* indicate sampling days

Spatiotemporal variations of in situ division rate (μ)

Table 1 shows the estimated minimum division rate, μ_{min} (Vaulot 1992), for each species of *Dinophysis* on each studied cycle, and the mean daily specific μ, T_c, and T_r using cytokinesis and sulcal list regeneration as terminal events (μf_{c+r}) and adjusting the data to a Gaussian function, as described in Reguera et al (2003) and Velo-Suárez et al. (2009), following the model of Carpenter and Chang (1988).

Early summer cycles

D.cf. acuminata In the three sampling stations, *D.* cf. *acuminata* division seemed phased during 3 and 4 June (2008) (Fig. 12a): Maximum frequency of paired cells (f_c) occurred within a very narrow (≤1 h) time before sunrise (0600 hours), and maximum frequency of recently divided cells (f_r) occurred within a similar little time (≤1 h) after sunrise. A second moderate frequency of paired cells was detected 30 min before sunset followed by a peak of recently divided cells 2 h after sunset. Two division times, over sunrise and sunset periods, were observed throughout this diel cycle sampling with moderate to high daily average specific division rates (0.30–0.50 day^{-1}) at stations S2, S3, and S1, respectively. Low to moderates minimum division rates μ_{min} were estimated (0.13 to 0.24 days^{-1}) in the stations S1, S2, and S3 (Table. 1). During mid-June diel cycle (15 and 16 June 2009), maximum

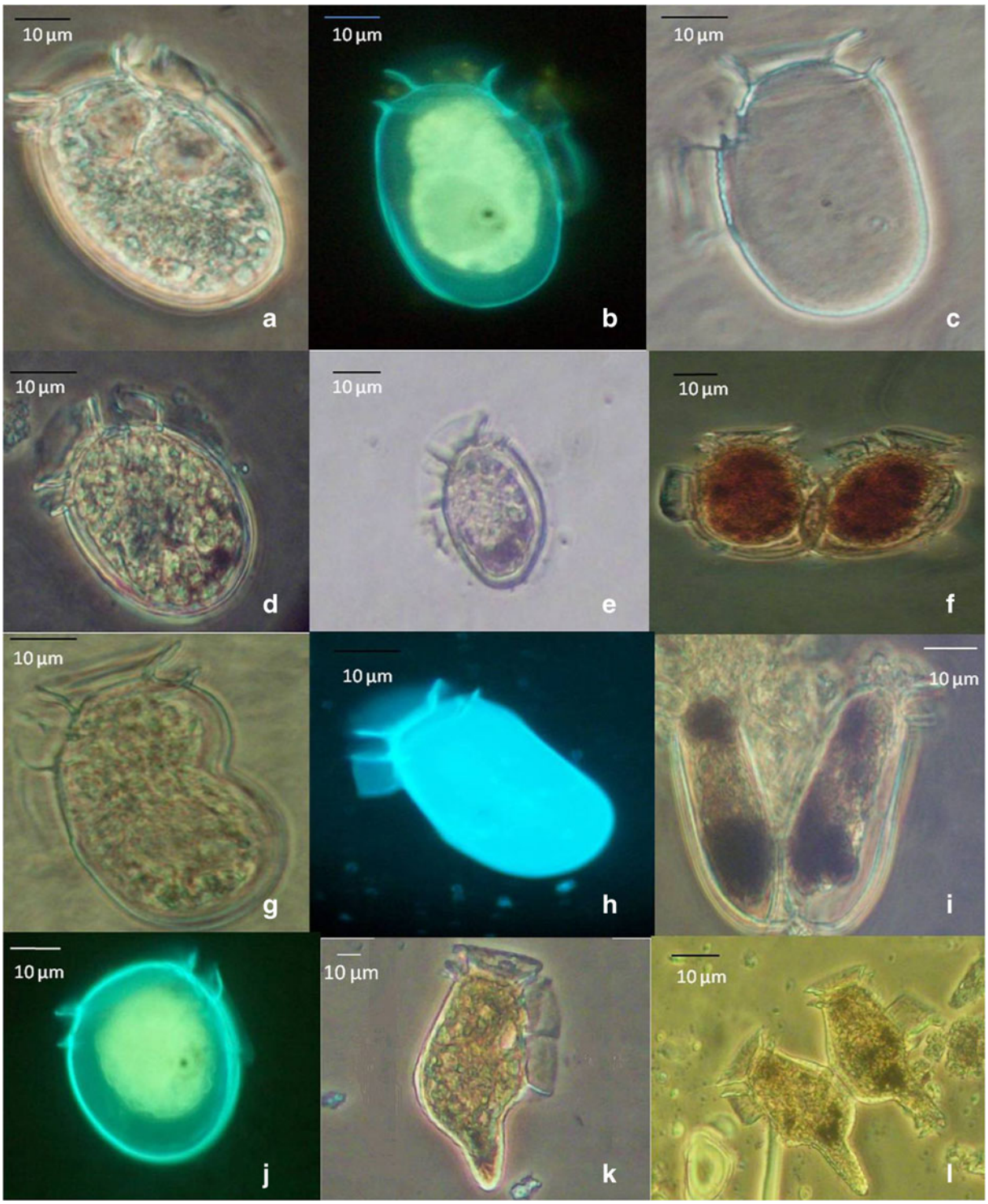

Fig. 11 *Dinophysis* species found in Punic harbors of Carthage from March 2008 to June 2010. **a**–**c** *D.* cf. *acuminata* from spring sampling. **a** Fully developed cell at the light microscope. **b** DAPI-stained cell. **c** Theca of recently divided cell showing the posterior half of the left sulcal list. **d**–**f.** *D.* cf. *acuminata* from summer sampling. **d**, **e** fully developed cell in light microscope. **f** Paired cells. **g**–**i** *D. sacculus*. **g** fully developed vegetative cell. **h** DAPI-stained recently divided cell showing the anterior half of the left sulcal list. **j** DAPI-stained cell of *D. rotundata*. **k** Fully developed cell of *D. caudata*. **l** recently divided pair of *D. caudata*

Table 1 *D.* cf. *acuminata* and *D. sacculus* Potentiel division rates (day^{-1}) during five diel cycles

Species	Date	Station	Temperature (°C)	Salinity (psu)	L/D (h)	T_c+T_r (h)	μ_{fc+r} (day^{-1})	μ_{min} (day^{-1})
D.acuminata	3–4 June 08	S 1	17–22.7	35–36.1	14.35/9.69	2.3	0.50	0.13
		S 2	18–25.7	36–37.1		3.2	0.33	0.13
		S 3	18–25.3	36–37.1		3.6	0.30	0.21
	2–3 November 08	S 1	15.5–23.5	35–36.1	10.37/13.65	2.1	0.39	0.12
		S 2	17–25.1	34–35		3.2	0.21	0.14
		S 3	18–24.4	34–35		3.5	0.20	0.15
	15–16 June 09	S 1	13.5–24.6	35–36	14.40/9.6	3.1	0.33	0.21
		S 2	14.1–25.2	35–36		3.8	0.37	0.22
		S 3	12–24.8	34–35		4.8	0.68	0.21
	30–31 October 09	S 1	13.5–23.5	34–35	10.41/13.59	5.1	0.16	0.14
		S 2	12.8–25.1	33.8–35		3.2	0.25	0.13
		S 3	18–24.4	33.8–35		2.3	0.33	0.15
	26–27 June 10	S 1	23.2–30	36–37	14.40/9.6	3.1	0.27	0.16
		S 2	24–31.2	36–37.3		4.1	0.22	0.17
		S 3	23.5- 30.7	36–37.1		2.1	0.39	0.22
D.sacculus	3–4 June 08	S 1	17 - 22.7	35–36.1	14.35/9.69	4.2	0.09	0.04
		S 2	18–25.7	36–37.1		2.3	0.23	0.18
		S 3	18–25.3	36–37.1		2.3	0.26	0.21
	2–3 November 08	S 1	15.5–23.5	35–36.1	10.37/13.65	2.1	0.09	0.02
		S 2	17–25.1	34–35		3.1	0.16	0.12
		S 3	18–24.4	34–35		3.1	0.19	0.16
	15–16 June 09	S 1	13.5–24.6	35–36	14.40/9.6	3.4	0.10	0.04
		S 2	14.1–25.2	35–36		4.2	0.21	0.21
		S 3	12–24.8	34–35		4.3	0.23	0.21
	30–31 October 09	S 1	13.5–23.5	34–35	10.41/13.59	2.3	0.16	0.10
		S 2	12.8–25.1	33.8–35		3.3	0.17	0.11
		S 3	18–24.4	33.8–35		4.2	0.14	0.10
	26–27 June 10	S 1	23.2–30	36–37	14.40/9.6	2.5	0.21	0.16
		S 2	24–31.2	36–37.3		2.6	0.35	0.15
		S 3	23.5- 30.7	36–37.1		2.4	0.32	0.10

L/D light/dark photoperiod, T_c, T_r duration of cytokinetic and the sulcal list regeneration cell phases respectively, μ_{min} minimum division rate according to the model of Vaulot (1992), μf_{c+r} potential growth rate calculated from cytokinetic and sulcal list regeneration phases applying the model of Carpenter and Chang (1988)

frequencies of paired cells occurred in a very short period of time (≤ 2 h) prior to sunrise and sunset simultaneously in the three sampling stations (Fig. 12b). Peaks of recently divided cells were observed from 30 min to 1 h after sunrise in the stations S2 and S3 but during sunrise in the station S1. These peaks appeared at dark period (<2 h after sunset) in the stations S1 and S2 and <1 h before sunset in station S3 (Fig. 12b). The highest value of the daily average specific division rate (0.68) was recorded in the station S3; however, this rate was lower than the half in the other stations (33–37 day^{-1}). Estimate of μ_{min} according to Eq. (3) was almost the same in all sampling stations (Table. 1).

In the end of June (26 and 27 June 2010), the cytokenesis lasted from 2100 to 0400 hours. The sulcal-list regeneration was longer, occurred from 2000 to 0830 hours. Maximum f_c were observed during dark period at 0300 hours in the station S1, at 2100 hours in S2 and at 0000 hours in S3. The highest

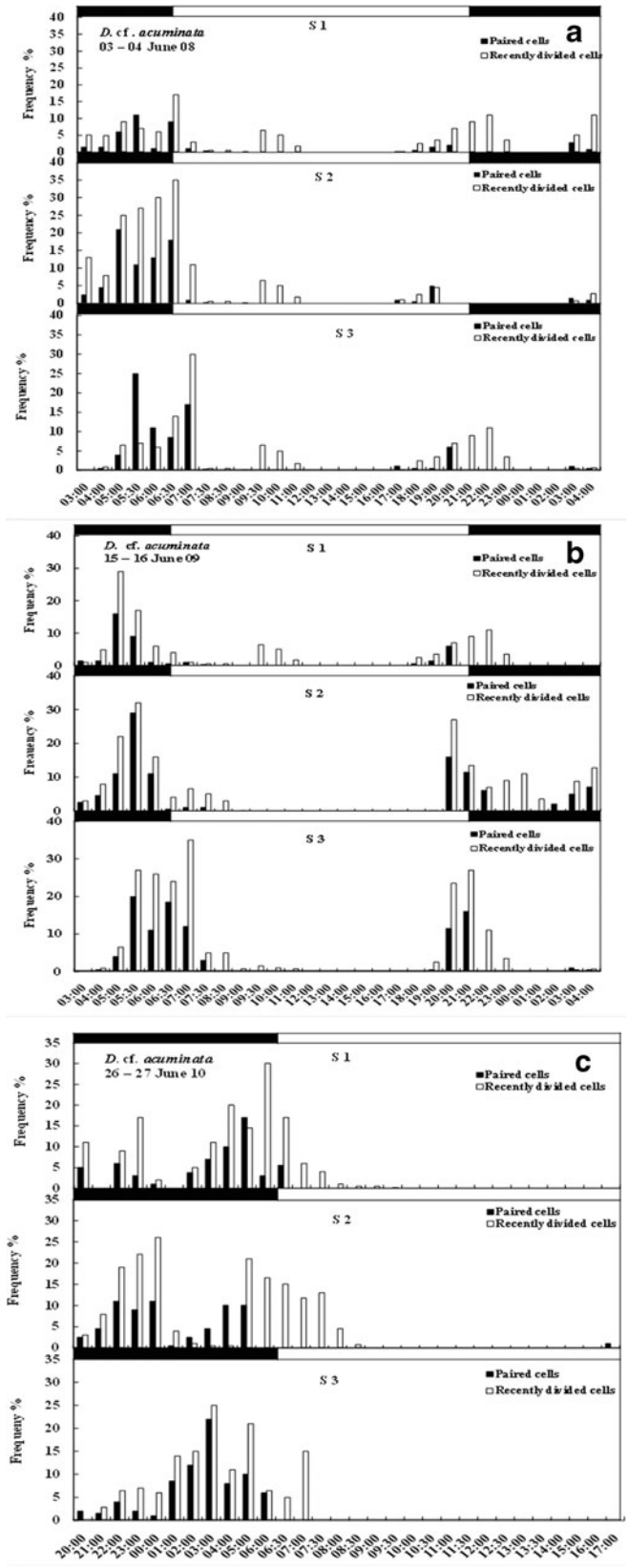

Fig. 12 Frequency distribution of paired and recently divided cells of *D.* cf. *acuminata* during early summer diel cycles (**a** 03–04 June 2008, **b** 15–16 June 2009, and **c** 26–27 June 2010). *Horizontal bar at top of graphs* indicates the period between sunset and sunrise

values of f_r occurred many hours before sunrise in the stations S2 and S3 but at sunrise in the station S1. Moderate's estimates μ (0.22–0.39 day^{-1}) and μ_{min} (0.16–0.22 day^{-1}) were observed during this diel cycle.

D. sacculus

At 3 and 4 June 2008, in all sampling stations, peaks of paired and recently divided cells recorded during or after sunrise (Fig. 13a). In station S1, the highest f_c was <5 %, which occurred 1 h before sunrise and the maximum of f_r (8 %) observed at 0700 hours (1 h after sunrise). Throughout this diel cycle, we estimated low minimum and mean daily divisions rates (0.04 and 0.09 day^{-1}) in the station S1 and moderate values (of μ_{min} and μ) in the other stations (Table. 1).

Division seemed strongly phased on 15 and 16 June 2009 (Fig. 13b). Cytokenesis and sulcal-list regeneration lasted from sunrise (0600 hours) to 1200 hours in all sampling stations. Peaks of paired cells occurred during sunrise in station S1 and 1 h after in the stations S2 and S3. Maximum of recently divided cells recorded 3 h after sunrise in the station S2 and S3 and after 90 min in the station S1. Few numbers of paired and recently divided cells appeared after 1800 hours in stations S2 and S3. We noted that *D. sacculus* shows low division rates in the early June cycle (Table. 1).

In the end of June (26 and 27 June 2010), maximum *D. sacculus* paired cells occurred during a time interval between 1 and 2 h prior to sunrise in all sampling stations. The highest values of f_r recorded during sunrise (06:00 h) in S1 and at 0730 hours in S2 and S3.Other peaks of f_c and f_r were observed, respectively, at 1400 and 1700 hours in S2 (Fig. 13c). Low μ_{min} (0.10–0.16 day^{-1}) values and moderate mean daily specific μ (0.21–0.35 day^{-1}) of *D. sacculus* characterized this diel cycle.

Autumn cycles

D.cf. acuminata A wide temporal distribution of frequencies of paired cells (f_c) and recently divided cells (f_r) during November 2008 (2 and 3 November) sampling in Punic harbors of Carthage (Fig. 14). The f_c and f_r maxima were observed after sunrise in the three sampling stations. The highest frequencies values varied between 11 and 16 %. Low estimated minimum growth rates (μ_{min}) (0.12–0.15 day^{-1}) and moderate mean daily specific μ (0.20–0.39 day^{-1}) of *D.* cf. *acuminata* were recorded in different stations during this diel cycle sampling.

During the sampling cycle of October 2009 (30 and 31 October), the terminal events of cell division lasted from 0600 to 1030 hours in the three sampling stations. The maximum frequency of paired cells was observed at 0730 hours in S1, at 0630 hours in S2 and S3. The maximum of f_r occurred at 0930 hours in S1, at 0800 hours in S2 and at 0730 hours in S3 (Fig. 14). The *D.* cf. *acuminata* division seemed to be highly phased during the 24 h of sampling on October 2009 with low in situ division rates (μ and μ_{min}; Table. 1).

D. sacculus

During November 2008 sampling, low f_r and f_c were detected in water sampling collected in the station S1 from 0800 to 1200 hours (Fig. 15), and very low minimum division rates were estimated (Table. 1). The highest frequencies of paired and recently divided cells occurred after sunrise in the sampling stations (Fig. 15). Low μ (0.12–0.16 day^{-1}) and μ_{min} (0.16–0.19 day^{-1}) were estimated in S2 and S3. In the end of October 2009, paired and recently divided *D. sacculus* cells appeared sporadically in S1 during this diel cycle sampling, no phased division was observed in this station. In contrast, strongly phased division was recorded in the other stations where the highest f_c occurred within a very narrow (≤120 min) time frame following sunrise and the f_r occurred 120 min later. However, the mean specific daily division rates were relatively low (Table 1).

Discussion

Low turbulence of water column stability can trigger dense populations of dinoflagellates (Margalef 1975). Dinoflagellate blooms occur in predominantly warm and stratified waters from late spring to early autumn (Smayda 1980). The combination of moderate upwelling and thermohaline stratification has been identified as an optimal condition for the development of harmful algal blooms (Tilstone et al. 1994) especially those of *Dinophysis* spp. (Reguera et al. 1995). Peak cell-abundance values of *D. sacculus* and *D.* cf. *acuminata*

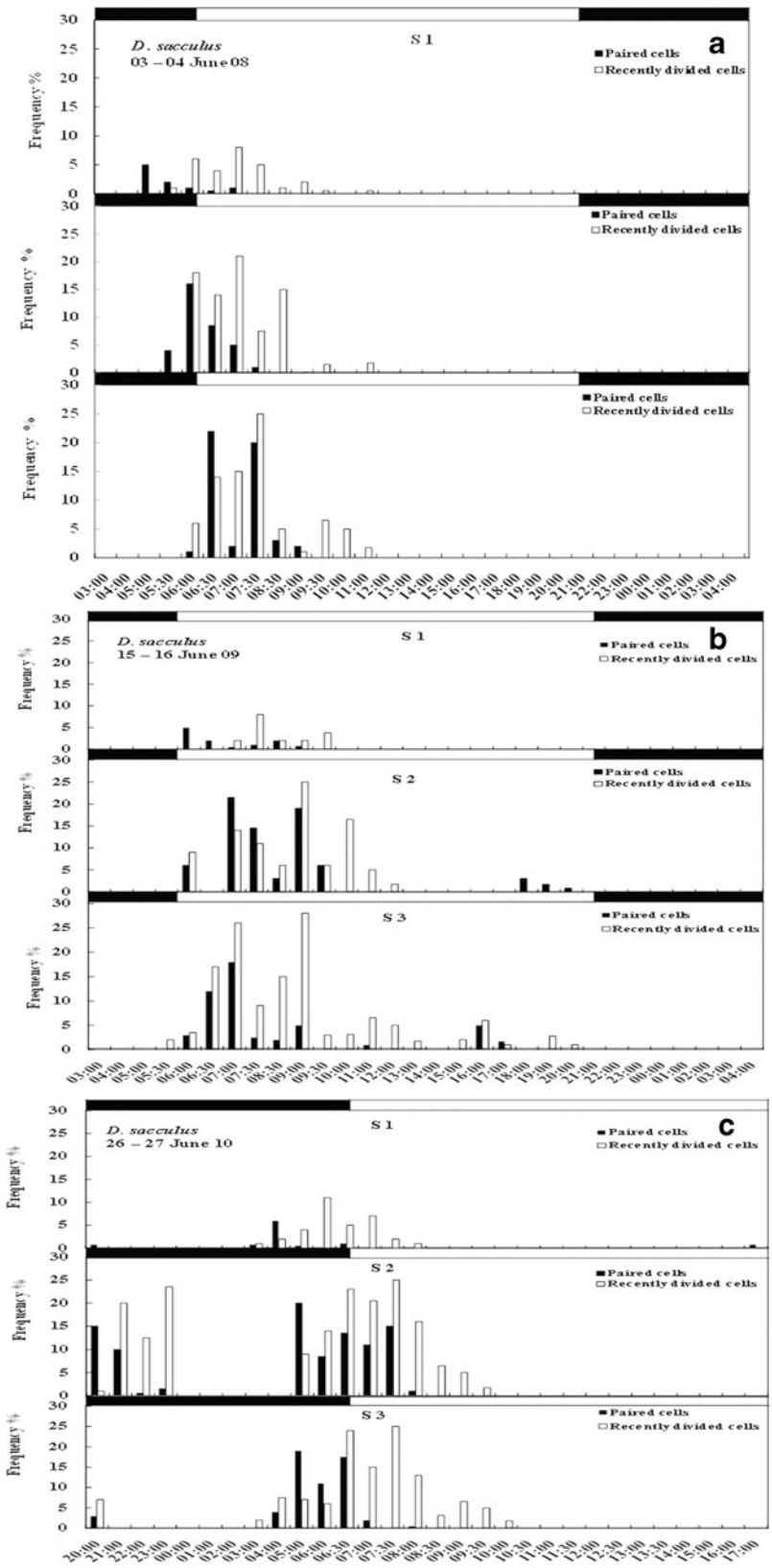

Fig. 13 Frequency distribution of paired and recently divided cells of *D. sacculus* during early summer diel cycles (**a** 03–04 June 2008, **b** 15–16 June 2009, and **c** 26–27 June 2010). *Horizontal bar at top of graphs* indicates the period between sunset and sunrise

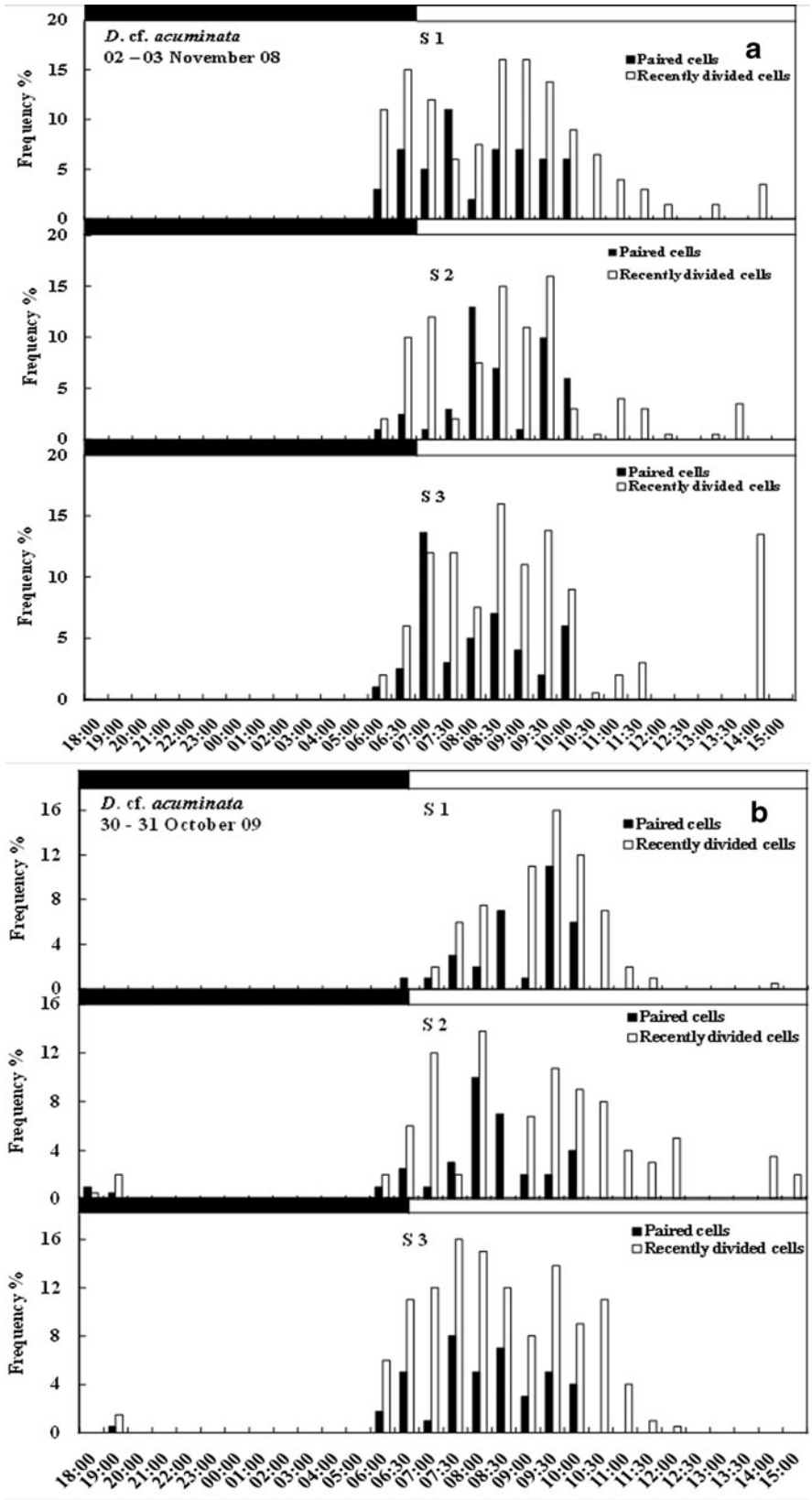

Fig. 14 Frequency distribution of paired and recently divided cells of *D.* cf. *acuminata* during autumn diel cycles (**a** 02–03 November 2008 and **b** 30–31 October 2009). *Horizontal bar at top of graphs* indicates the period between sunset and sunrise

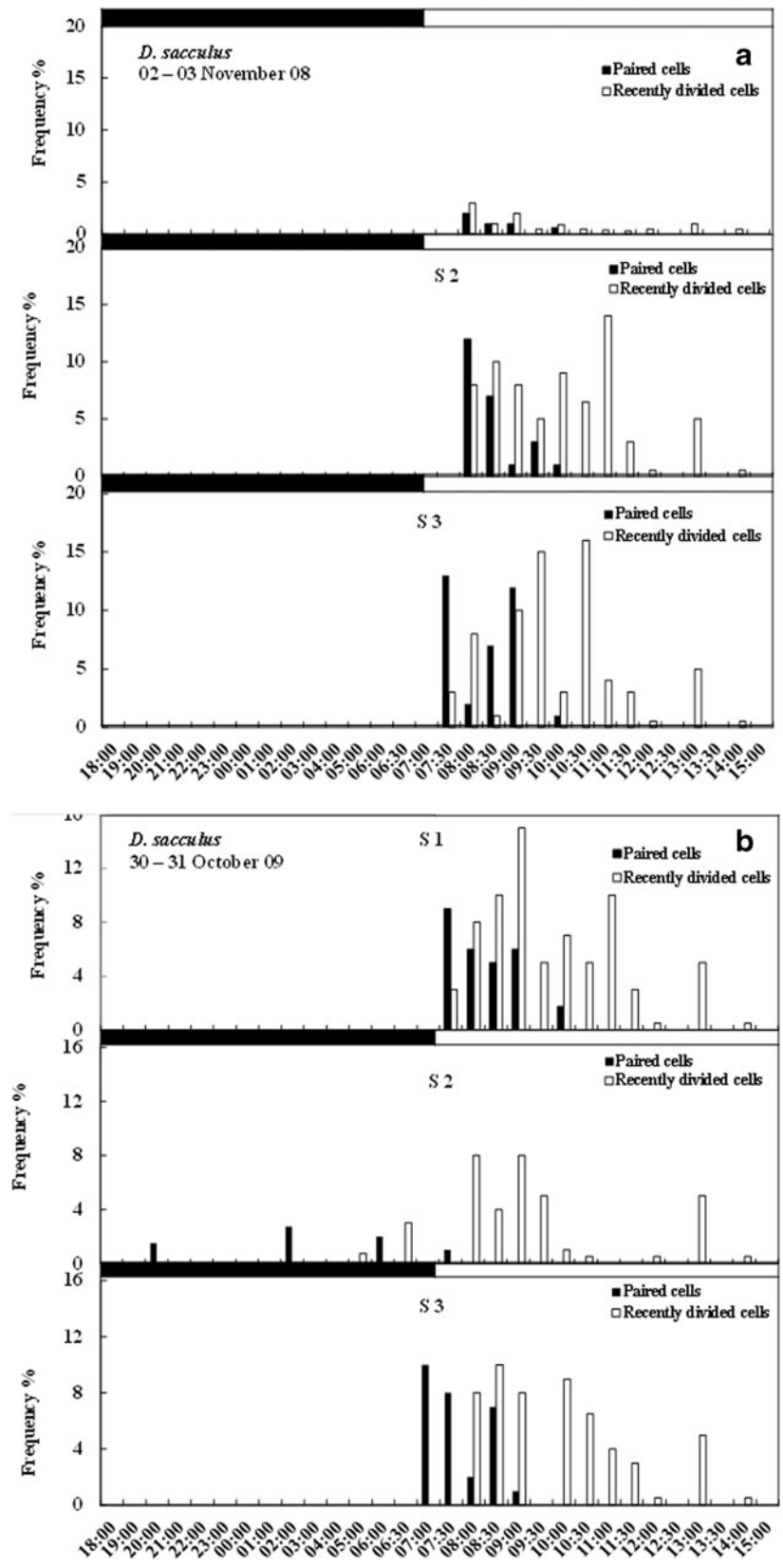

Fig. 15 Frequency distribution of paired and recently divided cells of *D. sacculus* during autumn diel cycles (**a** 02–03 November 2008 and **b** 30–31 October 2009). *Horizontal bar at top of graphs* indicates the period between sunset and sunrise

in Punic harbors of Carthage were recorded from March to June (2008–2009) and from September to November (2008–2009). In this shallow study area, the stratification of water column is not significant (Aissaoui et al. 2007) and cannot be the environmental condition promoting the increase in *Dinophysis* species. The seasonal occurrence of *D. sacculus* and *D.* cf. *acuminata* showed wide temporal distribution, indicating that these species show adaptability to environmental variations throughout the year, even if the highest abundances were associated with temperature (r=0.42, p< 0.05, n=126 for *D. sacculus* and r=0.38, p< 0.05, n=145 for *D. acuminata*) and salinity (r=0.34, p< 0.05, n=145 for *D. sacculus* and r= 0.29, p< 0.05, n=145 for *D. acuminata*) transitions. *D. sacculus* and *D.* cf. *acuminata* proliferations occur from early spring to early summer and in autumn at wide intervals of water temperature (13.70–30 °C) and salinity (33.60–40.10). The maximum densities of *D. sacculus* were registered usually in May (2008–2009) when water temperature higher than 22 °C and salinity higher than 35. Highest abundances of *D.* cf. *acuminata* were recorded especially on March 2009 during a lower water temperature and salinity (13.50 °C and 33.80, respectively). We concluded that these species tolerated wide variations of water temperature and salinity, but they require specific values which seem to stimulate their growth.

According to the morphological features used to discriminate between *D. sacculus* and *D. acuminata* cited in many taxonomic studies in the Mediterranean Sea (Jorgensen 1923; Lassus and Bardouil 1991; Giacobbe 1995; Zingone et al. 1998), *D.* cf. *acuminata* specimens found during our survey in Punic harbors of Carthage, appeared to be different to *D. sacculus*. However, specimens observed in early spring had an L/W ratio of 1.40 and showed a typical convex dorsal margin, and a rounded antapex appeared to be different to those found during early summer proliferation and has the same features of the original description by Claparède and Lachmann (1859). However, these spring specimens have similar features of those observed in the eastern Mediterranean Sea (Thermaikos Gulf) and identified as *Dinophysis ovum* (Koukaras and Nikoladis 2004; Dimitra et al. 2010). In addition, this species proliferate in Punic harbors of Carthage approximately in the same period as in Thermaikos Gulf (from February to April 2009).

Some species of *Dinophysis* have been observed to display in phase cell division in field populations from different temperate regions (Reguera et al. 2003; Velo-Suárez et al. 2009; Campbell et al. 2010). The results from in situ cell cycle studies presented here confirm that cellular division in *D.* cf. *acuminata* and *D. sacculus* under study was in phase with the exception of cell division in *D. sacculus* in the station S1 during October cycle. Two stages in *Dinophysis* cell division are easily identifiable using light microscopy. The first is of paired cells joined at the dorsal margin. The second is of recently divided cells, during which each daughter cell will have only one visible half of the left sulcal list (Fig.16). These morphological differences were consistent characters, and important key stages that can be used as "terminal events" in μ estimates according to the method of Carpenter and Chang (1988). Division time and minimum division rate are estimate only within intensive sampling through a period of cell division. Our results show that, in the beginning and in the middle of June cycles, maximum *D.* cf. *acuminata* paired cell occurred within a very narrow (≤1.5 h) time before sunrise in all the sampling stations. In contrast, maximum frequencies of recently divided cells were recorded after sunrise during morning division. This maintains the view that in this species, cellular division is activated by first light (Reguera et al. 2003). These results are in agreement with previous observations on fields of the same species in June cycle by Velo-Suárez et al. (2009). In the case of *D. sacculus*, we found that maximum frequencies of paired and recently divided cells occurred after sunrise in early summer and autumn cycles. It seemed that this species require a high light intensity for its cellular division since it is most likely a photosynthetic species (Larsen and Moestrup 1992). Therefore, Reguera et al. 2012 suggested that activating factors of cell division in *Dinophysis* that peaks in the transition from night to day, depending on the species, are somehow modulated in the deficiency of photoperiod.

In agreement with Reguera et al. (2003), peaks of *D.* cf. *acuminata* paired cells and recently divided cells occurred after sunrise during autumn cycles and maximum frequencies of dividing cells observed (f_c) were usually much lower than maximum frequencies of recently divided cells (f_r), these results were still observed with *D. sacculus* in our study. This implies that the separation of the two daughter cells was carried out in a time interval extremely fast. In addition, the linkage of the two daughter cells by megacytic bridge is very frangible and can be readily destroyed

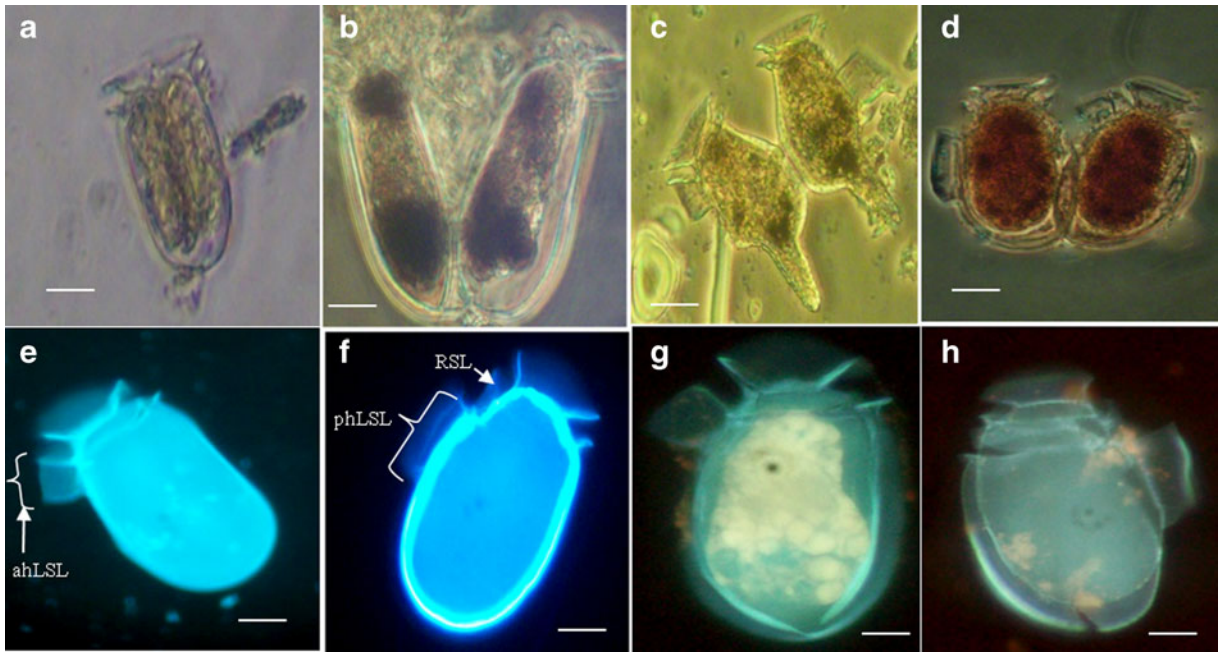

Fig. 16 Micrographis of two identifiable stages in *Dinophysis* cell division. **a**–**c** Paired daughter cell of *Dinophysis* joined at the dorsal margin. **a**, **b** *Dinophysis sacculus*. **c** *Dinophysis caudata*. **d** Paired cell of *Dinophysis acuminata* at the moment of separation. **e**–**h** Recently divided cells exhibiting incomplete development of the left sulcal list. **e** *D. sacculus* cell showing the anterior half of the left sulcal list (ahLSL). **f** *D. sacculus* cell showing the posterior half of the left sulcal list (phLSL) and the small right sulcal list (RSL). **g**, **h** Recently divided cell of *D. acuminata* showing the incomplete development of the LSL. *Scale bar* 10 μm

by sample manipulation (especially in net haul samples) and by strong fixatives such as formaldehyde. For this reason, Reguera et al. (2003) added the half of the number of recently divided cells to that of the dividing cells to obtain accurate estimates of the total frequency of division.

Our field observations in the Punic harbors of Carthage show that *D.* cf. *acuminata* and *D. sacculus* observed during different stages of their population growth and under distinct environmental conditions can exhibit a wide range of values in their intrinsic division rates, between practically zero and 0.68 day^{-1} (Table. 1). Values of μ_{min} were close to that of μf_{c+r} especially for *D. sacculus* during autumn cycles. The highest μ_{min} values (0.21 day^{-1}) of *D. sacculus* were recorded during mid-June cycle (15 and 16 June 2009), and the lowest values were observed in autumn cycles. In agreement with our observations, Garcés et al. (1997) recorded that in situ estimated division rates by mitotic index approach of *D. sacculus* were higher during early summer and lower at autumn. Despite the moderate μ_{min} estimated in the June sampling cycle, an increase in the *D. sacculus* population was observed the following day in Punic harbors of Carthage. After a high tide during night, water coming off Gulf of Tunis in the both basins harbors may highly concentrated of *D. sacculus*, which might be divided with important division rate inland the Gulf. According to Reguera et al. (2012), the coastal species of *Dinophysis* should not be considered as slow-growers. Rather, they seem to be excellent survivors, able to modulate their division rates, adopting extremely low division rates to persist in the water column in the absence of prey. This can explain the wide temporal distribution of *Dinophysis* species in Punic harbors of Carthage.

Higher values of daily average specific division rate obtained for *D.* cf. *acuminata* during June cycles varied between stations sampled in coastal waters of the Gulf of Tunis (Table. 1). During June cycle, Velo-Suárez et al. (2009) observed that both integrated values of μ (the whole water column where *D. acuminata* was found) and those estimated at a specific depth where conditions are optimal were of similar magnitude (0.40 and 0.46 day^{-1}) and suggested that *D. acuminata* was

dividing at the same rate throughout the whole water column, and high densities of this species observed at different depths were caused by advection and vertical migration and not by higher division rates. Then, it can be said that low depths in Punic harbors of Carthage have no influence on the estimates of in situ division rates of *D.* cf. *acuminata*. Maximum values of division rates found during mid-June cycles for *D. sacclus* and *D.* cf. *acuminata* were observed during this diel cycle and showed that these species seemed to have the same requirement of temperature, salinity, and light/dark cycle for dividing. In fact, from 15th to the end of June, light period is the longest in the year (Table 1). Reguera et al.(2012) considered that estimated growth rate in *Dinophysis* spp. cultures include losses due to natural death (cell lysis) as equivalent to μ_{gross} (μ_{min}) during the exponential growth phase since other loss rates are negligible. In laboratory culture of *D. acuminata* feeding on its live prey *Myrionecta rubrum* and under continuous illumination, Park et al. (2006) recorded high values of μ (0.95 day^{-1}). However, during June cycle (26 and 27 June 2010), *D.* cf. *acuminata* divided in the Punic harbors of Carthage during the dark period with moderate division rates (Table. 1); it might be that the low intensity of moonlight triggered this cell division. Indeed, Kim et al. (2008) concluded that *D. acuminata* in laboratory culture continues to grow in the light in the presence of the prey and it fails to grow in darkness, despite the availability of prey, suggesting that this species that requires both light and prey for long-term survival and defining it as an obligate mixotroph. In fact, based on the same kind of mixotrophic nutrition, several *Dinophysis* species have subsequently been established in culture. These include *D. caudata* (Nishitani et al. 2008a; Park et al. 2008), *Dinophysis fortii* (Nagai et al. 2008), *Dinophysis infundibulus* (Nishitani et al. 2008b), *Dinophysis acuta* (Jaén et al. 2009), and *Dinophysis tripos* (Rodríguez et al. 2012).

Although they proliferated in the same period and/or simultaneously, *D.* cf. *acuminata* and *D. sacculus* seemed to have different strategies and patterns of cell division. This can be explained by their different ecological behavior; many studies (Jacobson and Anderson 1994; Janson 2004; Park et al. 2006) have shown that *D. acuminata* is an obligate mixotroph, but the mixotrophy behavior has not been confirmed in *D. sacculus*. Caroppo (2001) suggested that *D. sacculus* in Mediterranean Sea (Varano Lagoon) seemed to be autotrophic.

Conclusion

In this first study in the Punic harbors of Carthage, coastal *Dinophysis* species appeared to be permanent species of this semienclosed and shallow ecosystem and tolerate a wide variation of temperature and salinity. Moderate to high in situ divisions rates of *D.* cf. *acuminata* were estimated by the postmitotic index method and showed that this species can divided in low depths, but it is difficult to determine if increased numbers found in the whole column water are due to in situ division rate, physical accumulation or combination of both. However, low to moderate in situ division rates of *D. sacculus* were recorded despite the higher abundances of this species. Indeed, the near future knowledge of the kind of nutrition and establishment of the culture of this species can help to explain the scenario of which *D. sacculus* occurred with high concentrations and low division rates.

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